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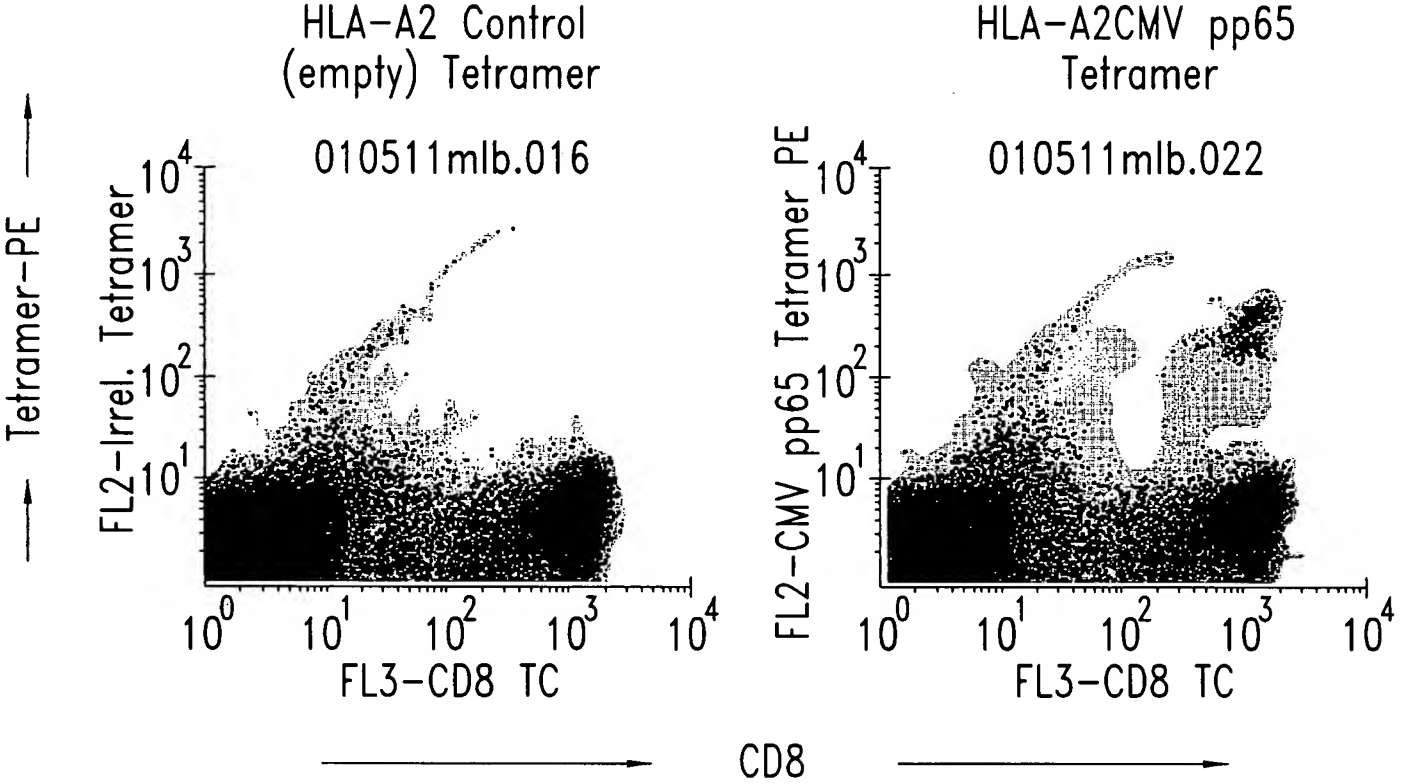
Serial No. 10/729,822 Docket No. 980034.42201
Inventor(s): Ronald Berenson et al.
Express Mail No. EV336654419US "REPLACEMENT SHEET"

Flow Cytometric Analysis of HLA-A2+ Donor T Cells for HLA-A2 CMVpp65+ T Cells: Day 0 of Culture

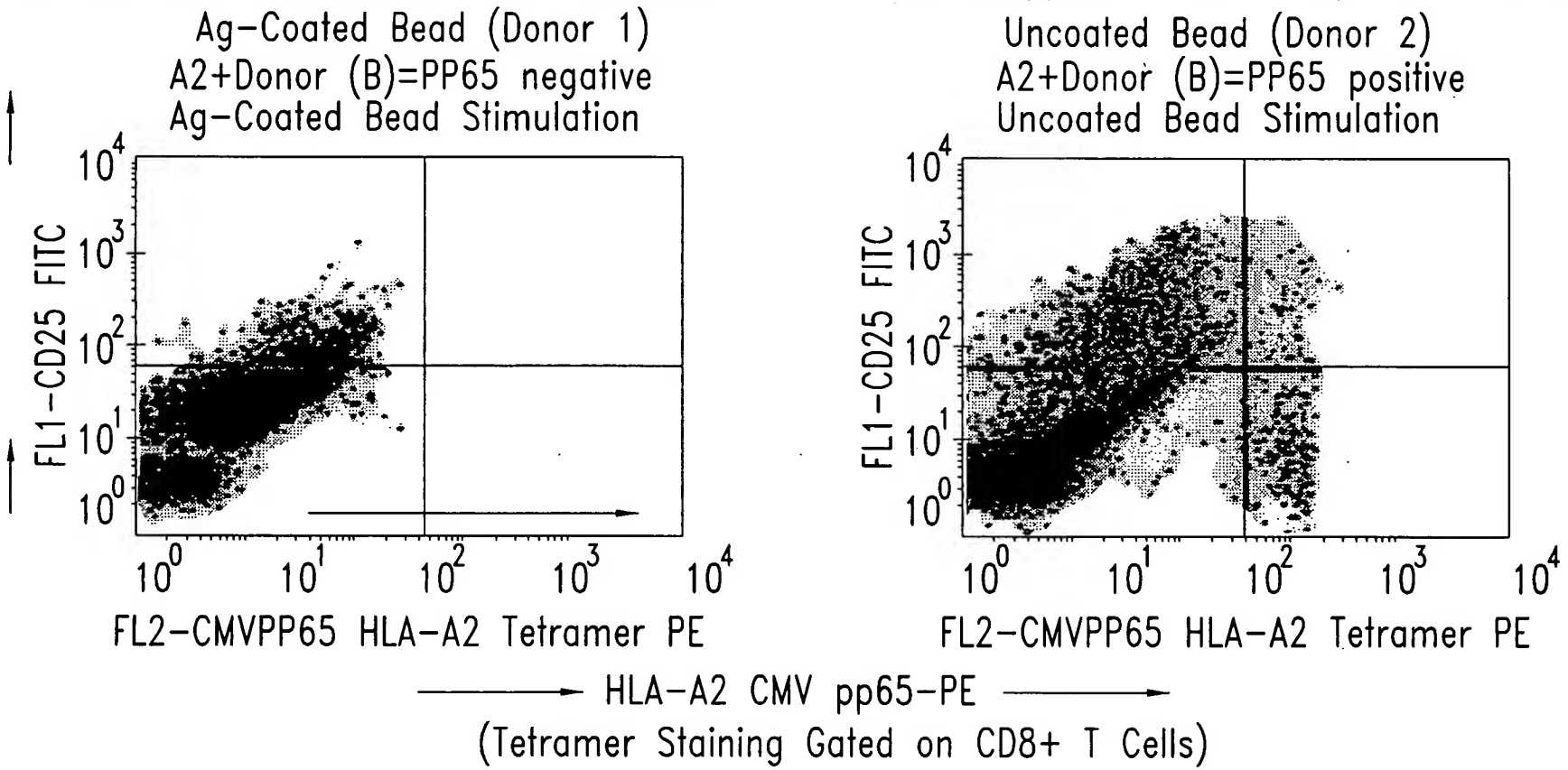


Human PBMC were screened for HLA-A2 positivity. HLA-A2+ donors were screened with control (empty) HLA-A2 tetramers and CMVpp65 loaded tetramers. In the donor shown above, approximately 3% of the CD3+CD8+ express TCR specific for HLA-A2 CMVpp65.

FIG. 1A

FIG. 1B

Flow Cytometric Analysis of CD25 Expression on HLA-A2 CMVpp65+ T Cells: Day 10 of Culture

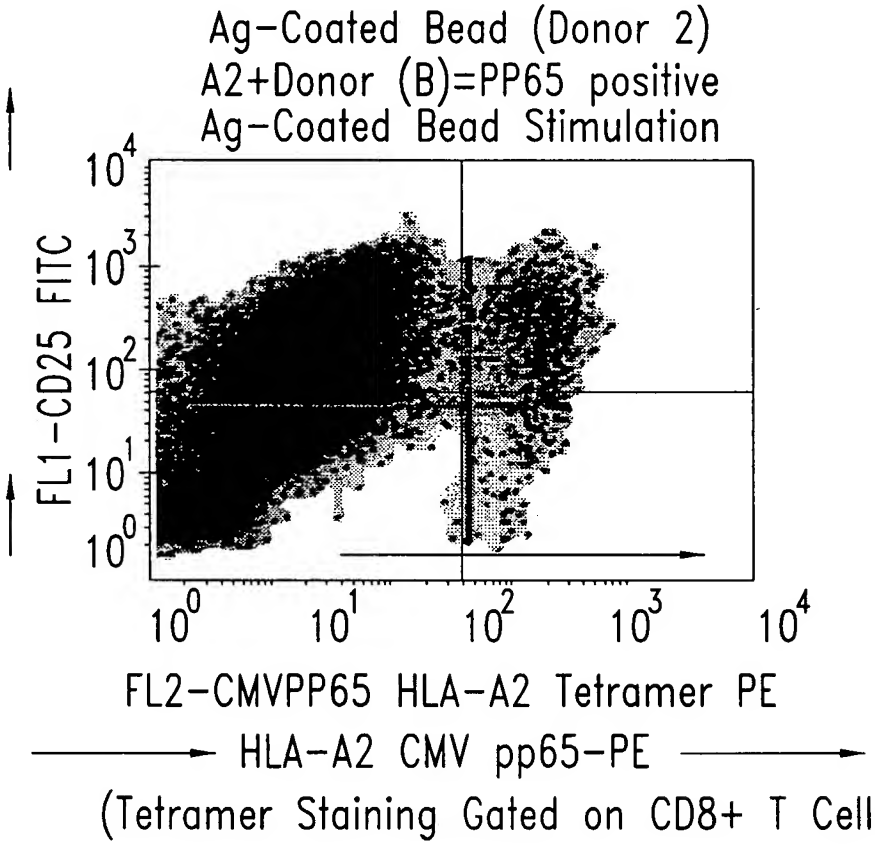


PBMC were activated with CMV antigen (coated onto paramagnetic beads) and by day 10 of culture, many cell are shown to be CD25 (IL-2R) positive, and all of the HLA-A2 CMVpp65+ T cells are expressing high levels of CD25, indicating activation (FIG. 2C). Controls include the same donor cells treated with uncoated (antigen-negative) beads (FIG. 2B), or an HLA-A2+ donor (donor 1) that did not show detectable tetramer+ cells at day 0 and was serologically negative for CMV (FIG. 2A). These data indicate that tetramer approaches can be effectively used to track antigen-specific T cells and their relative state of activation.

FIG. 2A

FIG. 2B

Flow Cytometric Analysis of CD25 Expression on HLA-A2 CMVpp65+ T Cells: Day 10 of Culture



PBMC were activated with CMV antigen (coated onto paramagnetic beads) and by day 10 of culture, many cell are shown to be CD25 (IL-2R) positive, and all of the HLA-A2 CMVpp65+ T cells are expressing high levels of CD25, indicating activation (FIG. 2C). Controls include the same donor cells treated with uncoated (antigen-negative) beads (FIG. 2B), or an HLA-A2+ donor (donor 1) that did not show detectable tetramer+ cells at day 0 and was serologically negative for CMV (FIG. 2A). These data indicate that tetramer approaches can be effectively used to track antigen-specific T cells and their relative state of activation.

FIG. 2C

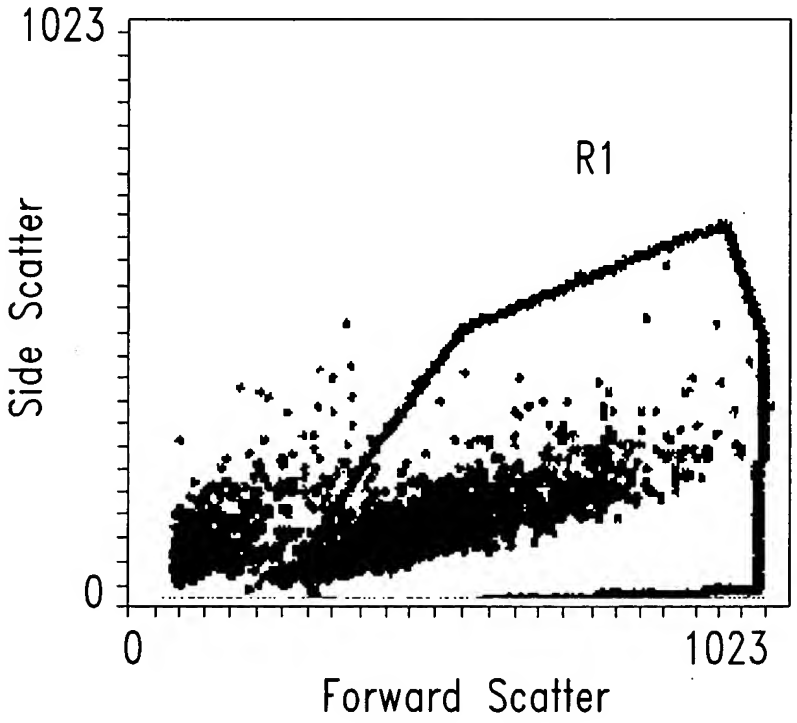


FIG. 3A1

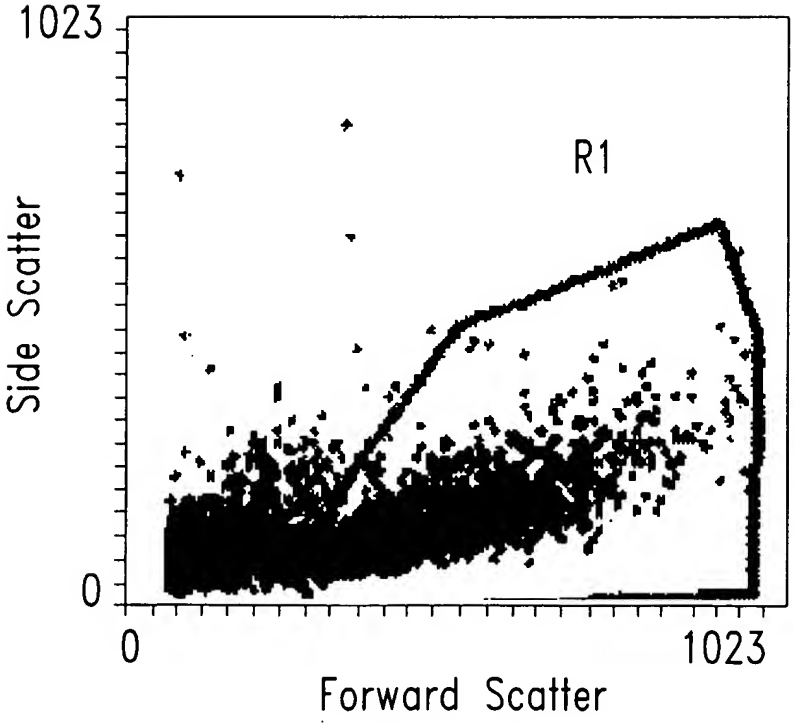


FIG. 3B1

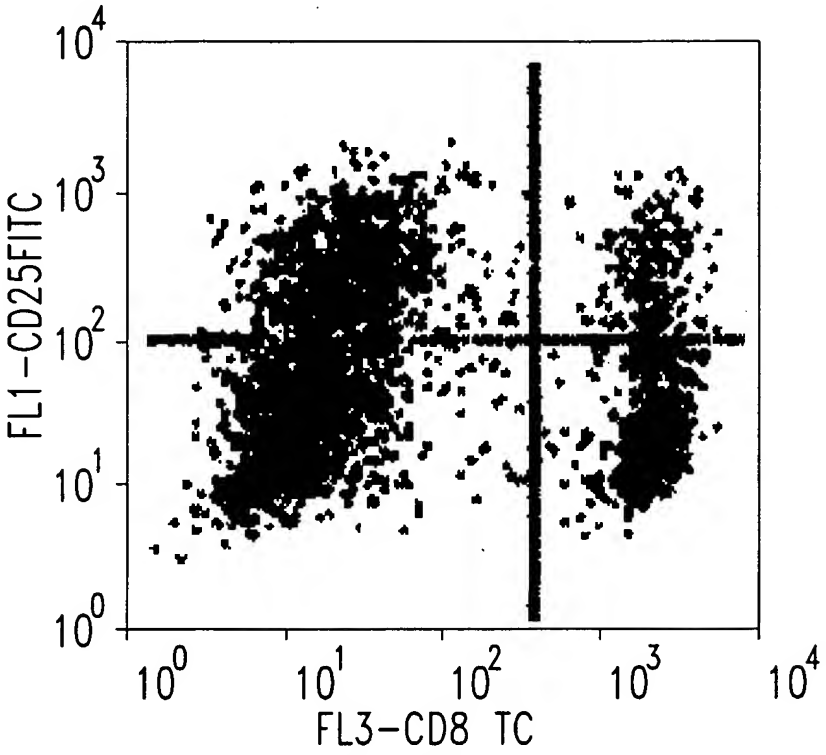


FIG. 3A2

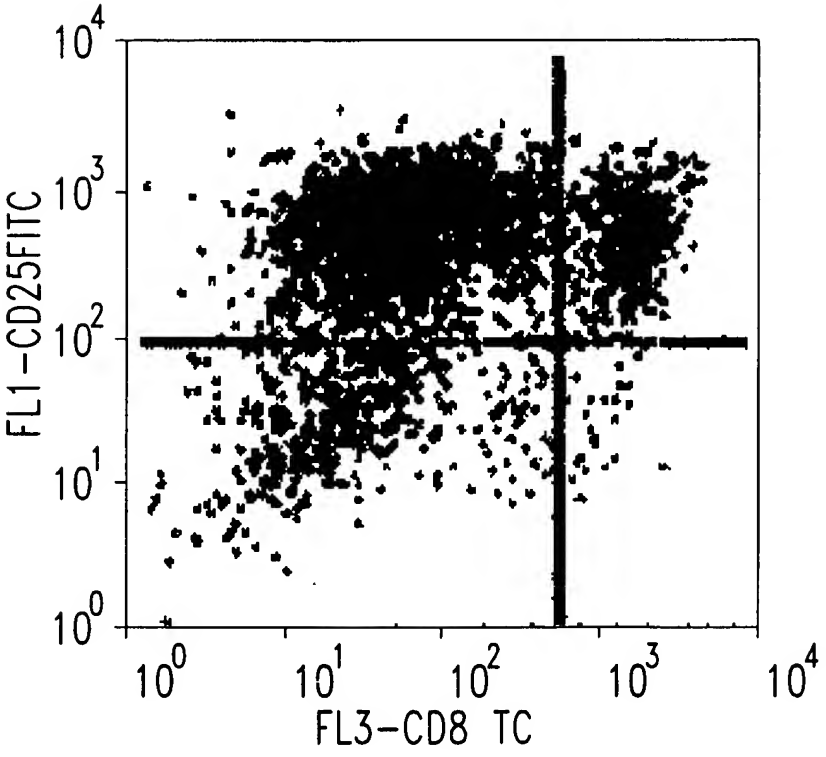


FIG. 3B2

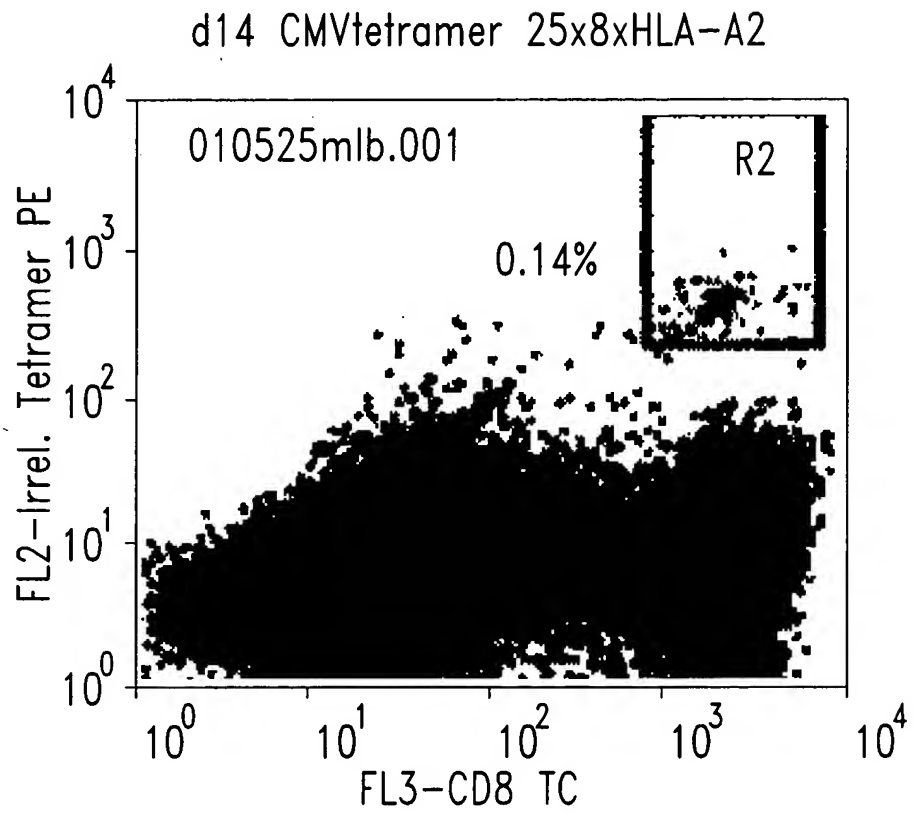


FIG. 3A3

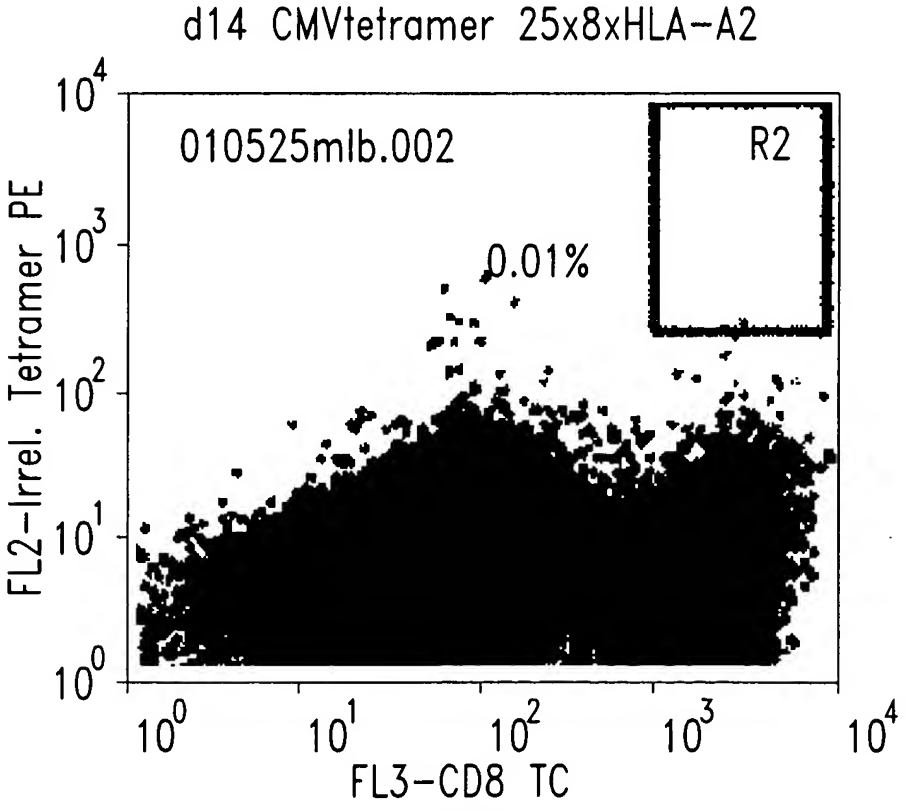


FIG. 3B3

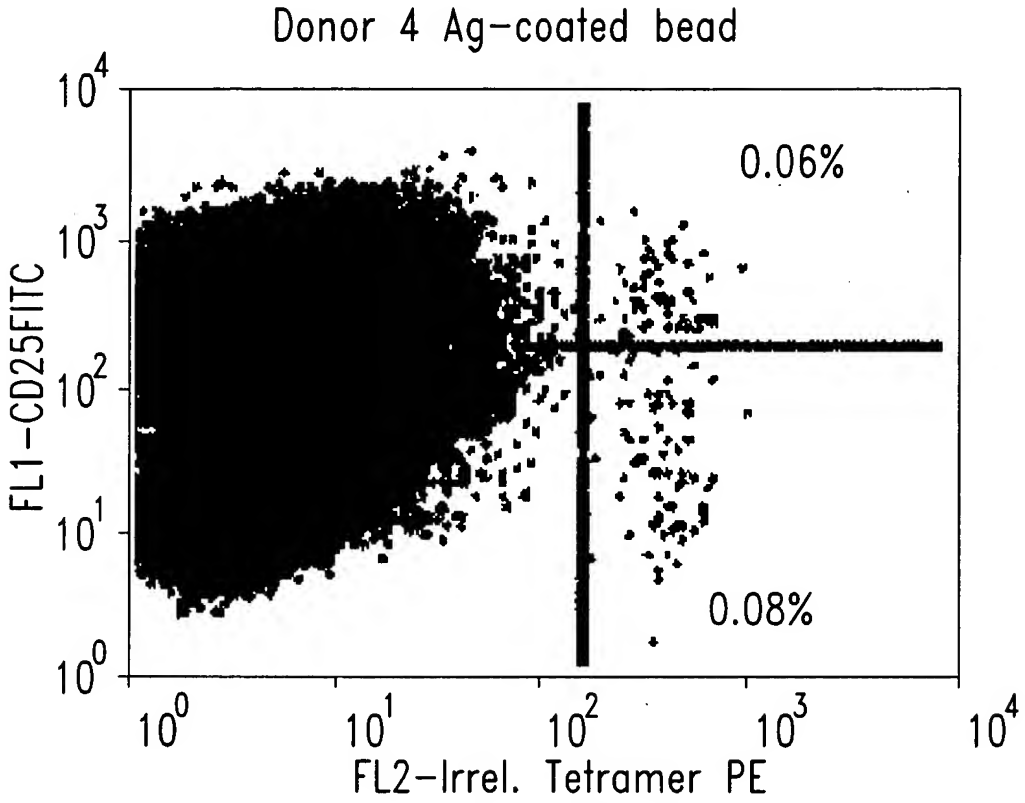


FIG. 3A4

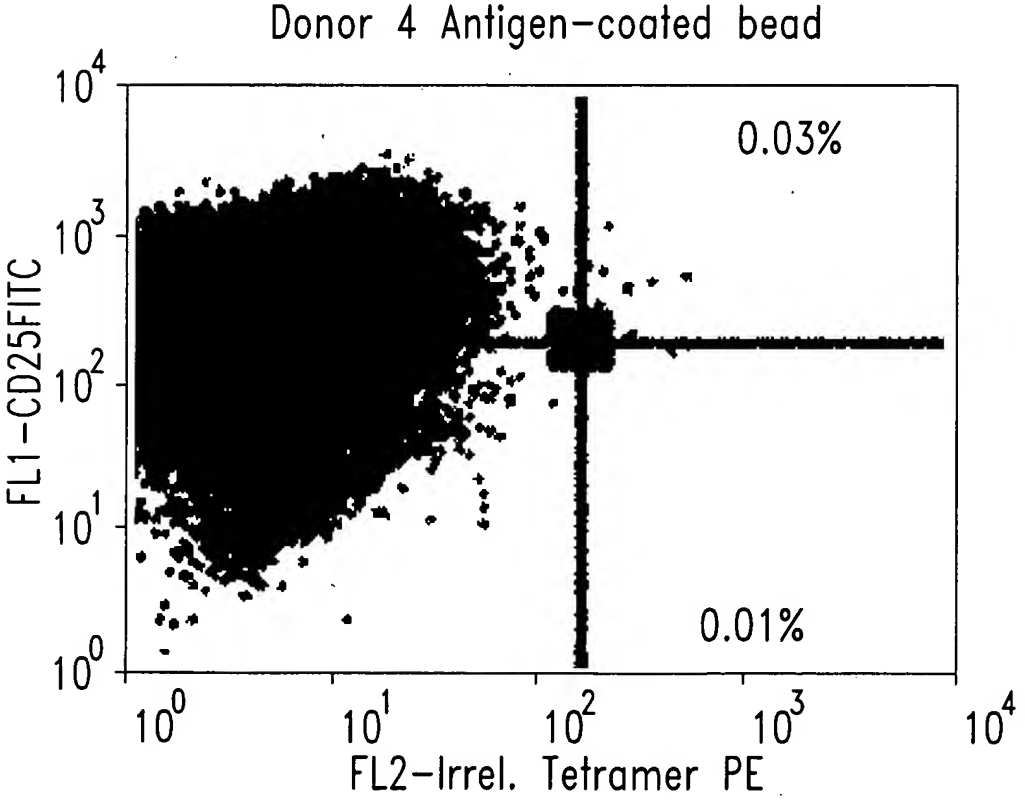


FIG. 3B4

Mixing Xcellerated T Cells with Autologous B-CLL Leukemic Cells Results in the Rapid Upregulation of Key Immunological Effector Molecules Day 12 Xcellerated T Cells Co-cultured 24 hours with autologous leukemic B Cells

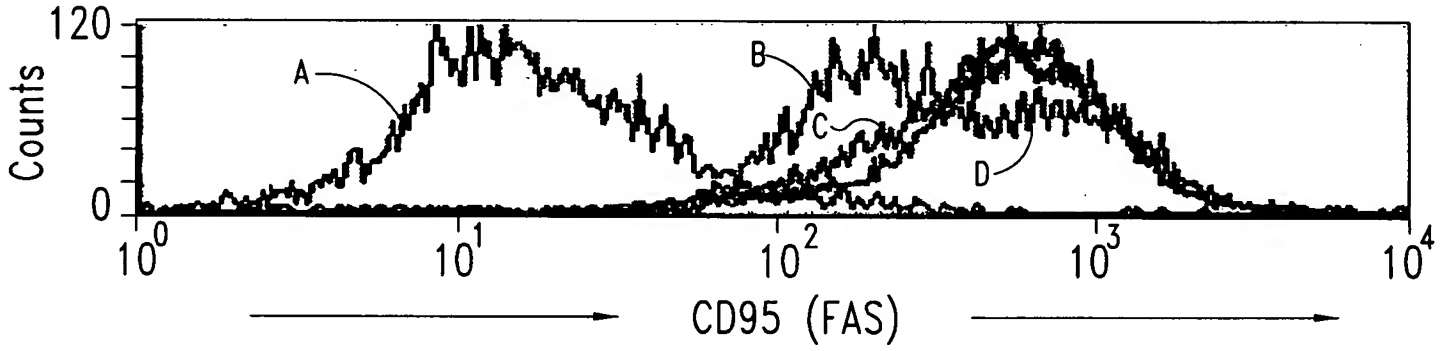


FIG. 4A

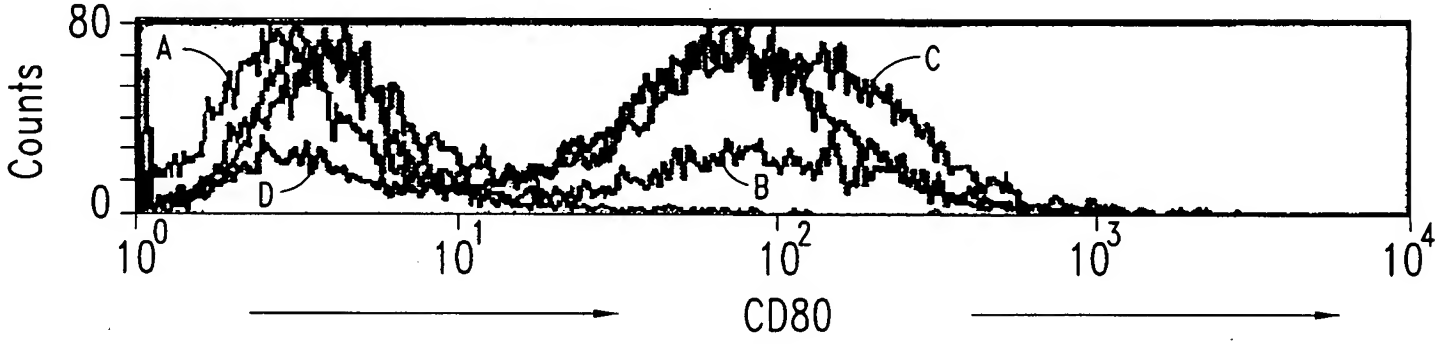


FIG. 4B

A= Leukemic B Cells Alone	B= Leukemic B Cells + Xcellerated T Cells T:B ratio=0.3:1	C= Leukemic B Cells + Xcellerated T Cells T:B ratio=1:1	D= Leukemic B Cells + Xcellerated T Cells T:B ratio=3:1

Mixing Xcellerated T Cells with Autologous B-CLL Leukemic Cells Results in the Rapid Upregulation of Key Immunological Effector Molecules Day 12 Xcellerated T Cells Co-cultured 24 hours with autologous leukemic B Cells

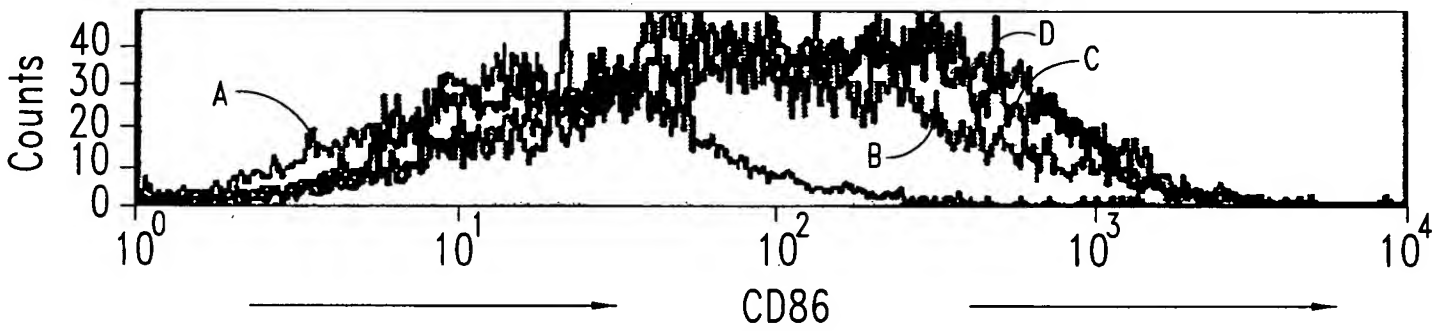


FIG. 4C

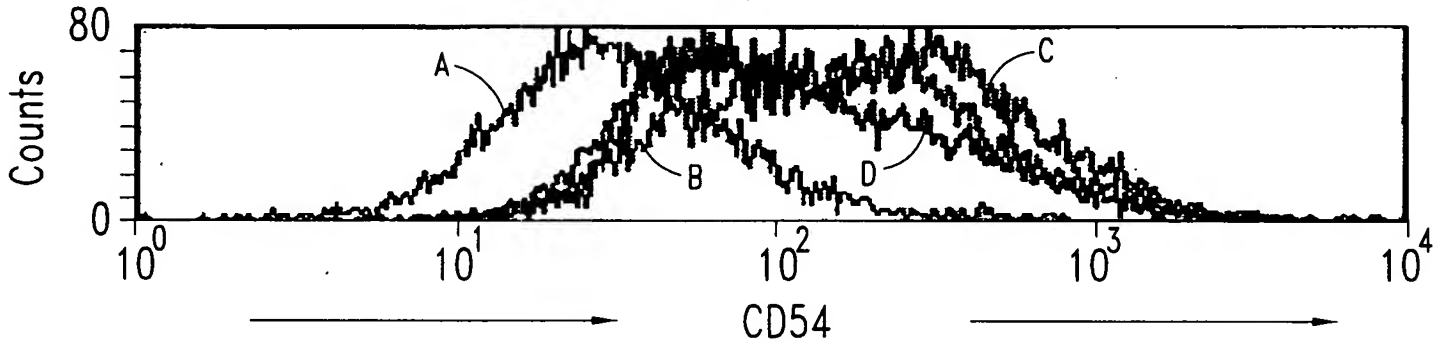


FIG. 4D

A= Leukemic B Cells Alone	B= Leukemic B Cells + Xcellerated T Cells T:B ratio=0.3:1	C= Leukemic B Cells + Xcellerated T Cells T:B ratio=1:1	D= Leukemic B Cells + Xcellerated T Cells T:B ratio=3:1

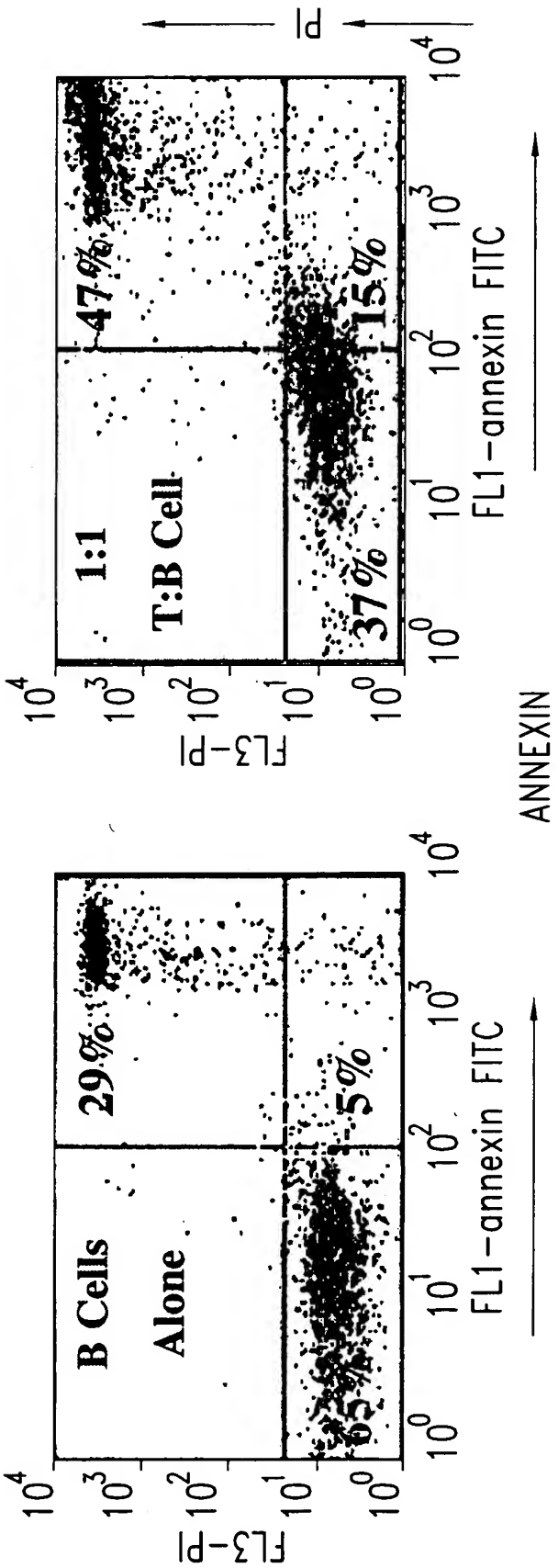


FIG. 5A

FIG. 5B

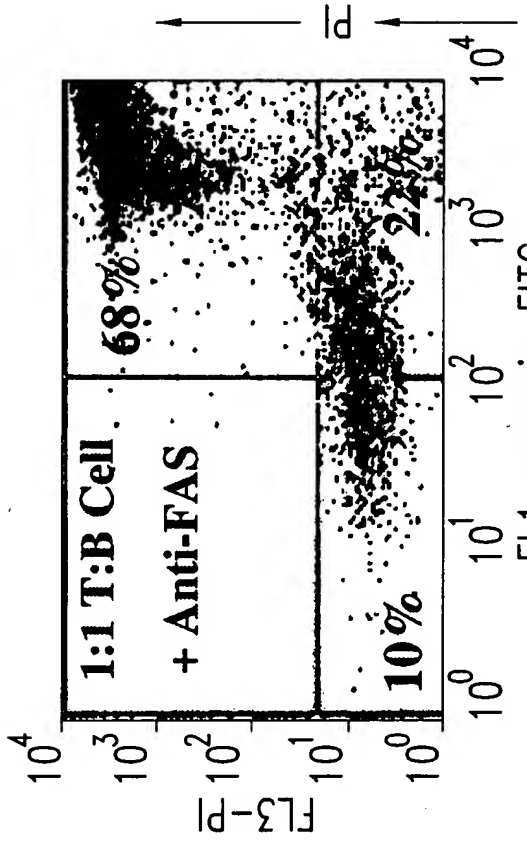


FIG. 5D

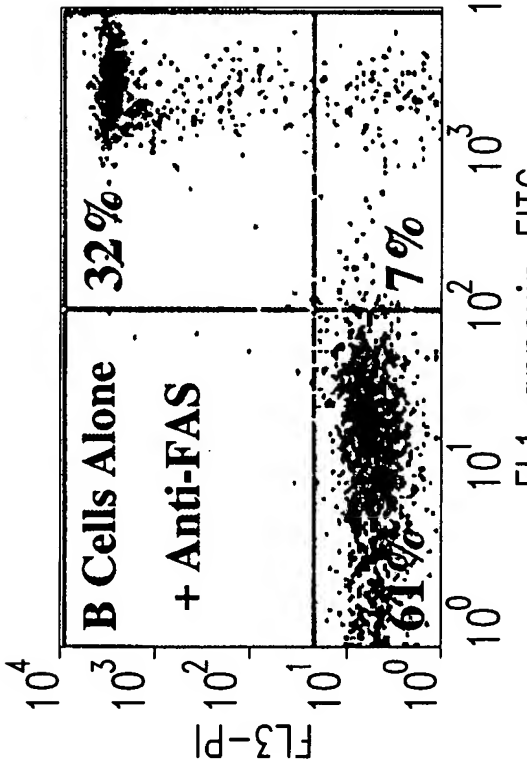


FIG. 5C

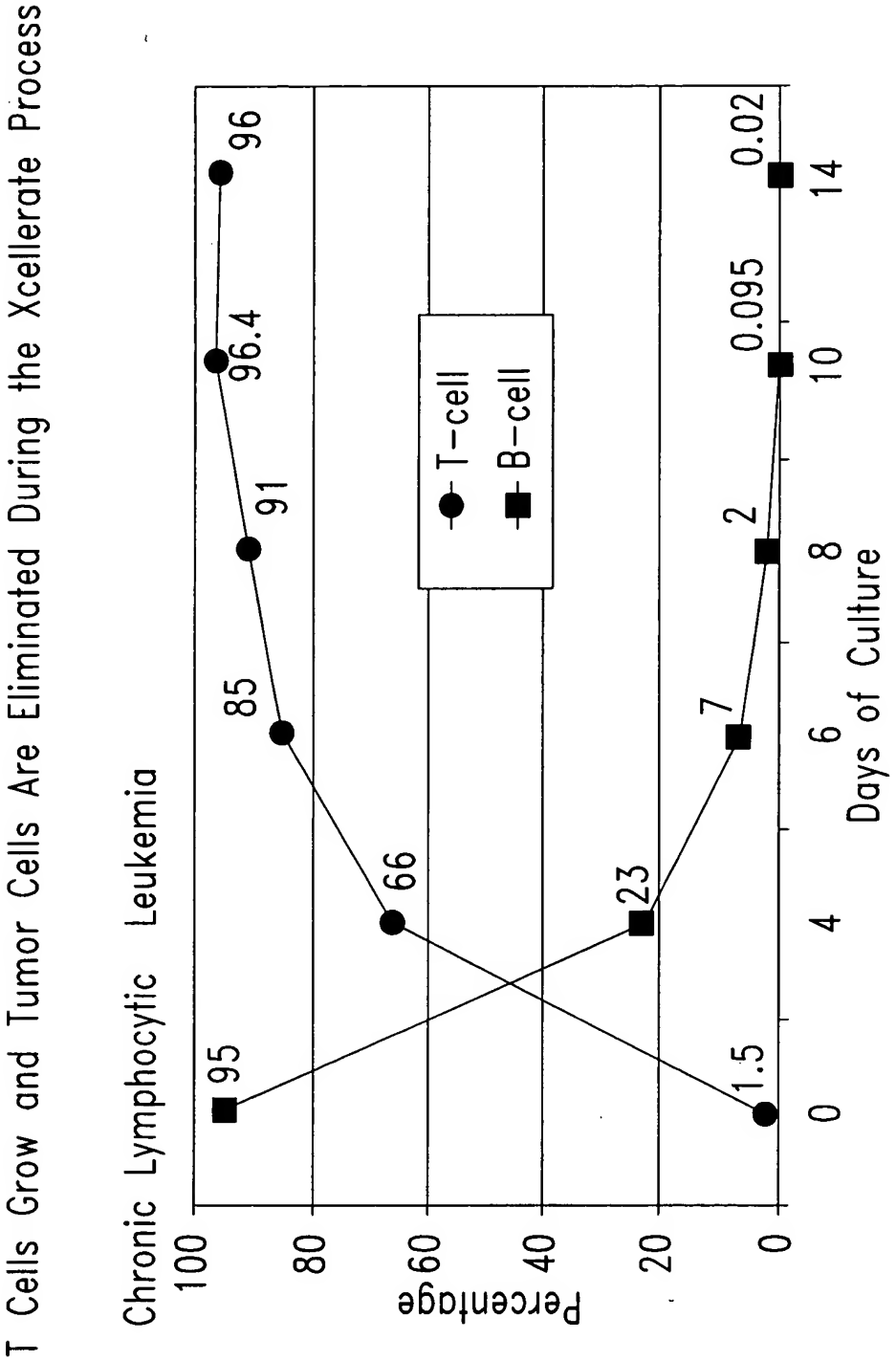


FIG. 6

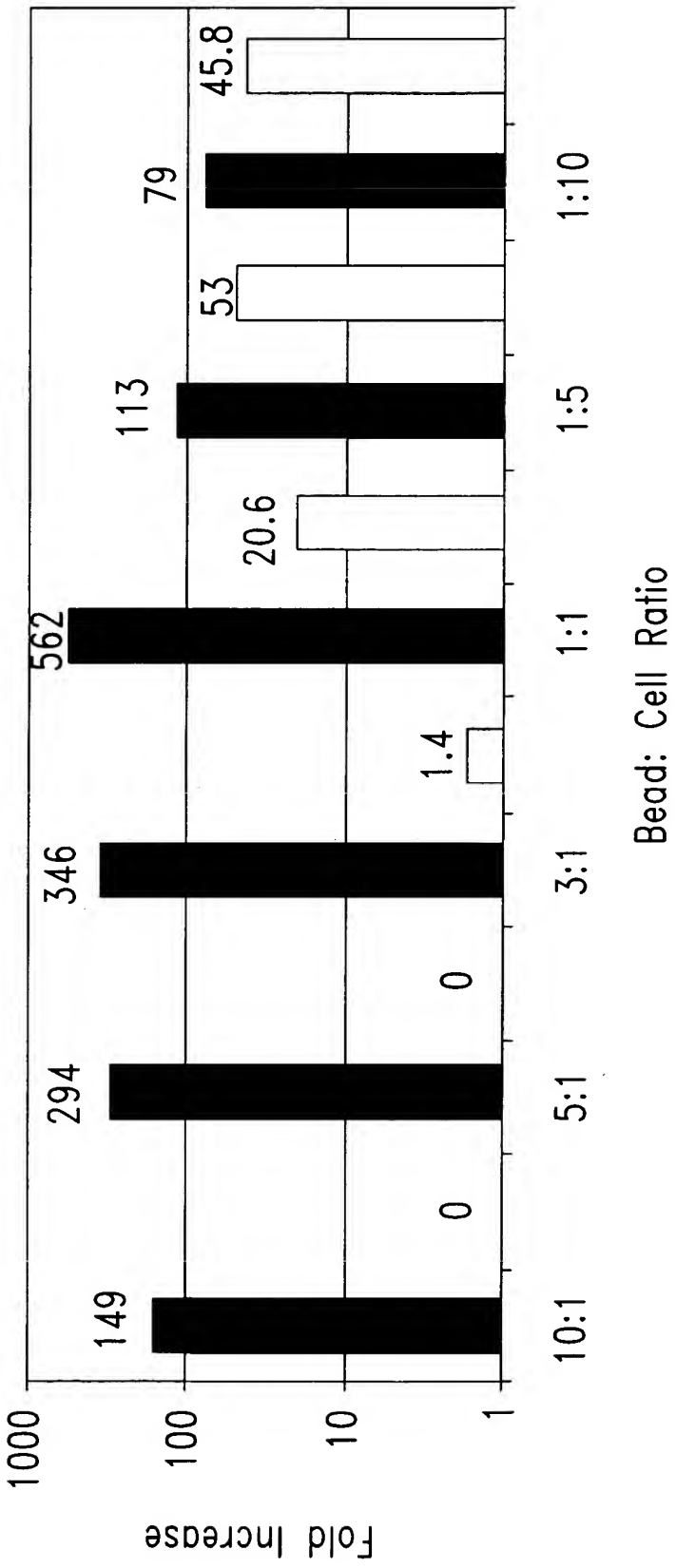


FIG. 7